U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
PB-286 998

Stress Ethylene Evolution A Measure of Ozone Affects on Plants

(U.S.) Corvallis Environmental Research Lab, OR

7 May 76

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3
EPA-600/J-76-079	JOURNAL ARTICLE	PB 286998

4. TITLE AND SUBTITLE	5. REPORT DATE
Stress Ethylene Evolution: A Measure of Ozone Affects On Plants	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
David T.Tingey, Carol Standley, and Richard W. Field	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Enviromental Researc[h] Laboratory, Corvallis EPA ORD Corvallis, Oregon	1AA602 11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
same	journal articl 14. SPONSORING AGENCY CODE EPA/ORD

15. SUPPLEMENTARY NOTES

Published in Atmosphere Enviroment Vol. 10 pp.969-974, 1976

16. ABSTRACT

To determine if ethylene evolution by plants is correlated with the ozone stress, a range of plants species and cultivars was exposed to varying ozone concentrations. Following exposure, the plants were encapsulated in plastic bags and incubated for up to 22h. The stress-induced ethylene that accumulated in the bag was monitored and correlated with the effects of ozone on plants. The rate of stress-induced ethylene production(slope parameter B) as a function of ozone concentration was used as a measure of plant sensitivity. Applying this ranking scale, ponderosa pine, eucalyptus and soybean were the most sensitive species; holly, squash and marigold were the least sensitive. There was a good correlation between ozone induced stress ethylene production and foliar injury. However, the coefficient of variability associated with the ethylene determination was substantially less than with the visual injury estimate. The production of stress-induced ethylene generally lasted for less than 48h. following exposure. The measurement of stress ethylene appeared to be a fast, reliable,sensitive and reproducible technique to measure ozone stress on plants.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Ethylene ozone		07/B,C

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
	unclassified	8
	20. SECURITY CLASS *(This page)*	22. PRICE
	unclassified	A02-A01

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA-600/J-76-079
JOURNAL ARTICLE

Atmospheric Environment Vol. 10, pp. 969–974. Pergamon Press 1976. Printed in Great Britain.

STRESS ETHYLENE EVOLUTION: A MEASURE OF OZONE EFFECTS ON PLANTS

DAVID T. TINGEY*, CAROL STANDLEY and RICHARD W. FIELD
Corvallis Environmental Research Laboratory, Environmental Protection Agency, 200 SW 35th St., Corvallis, OR, U.S.A.

(*First received* 12 *February* 1976 *and in final form* 7 *May* 1976)

Abstract—To determine if ethylene evolution by plants was correlated with ozone stress, a range of plant species and cultivars was exposed to varying ozone concentrations. Following exposure, the plants were encapsulated in plastic bags and incubated for up to 22 h. The stress-induced ethylene that accumulated in the bag was monitored and correlated with the effects of ozone on plants. The rate of stress-induced ethylene production (slope parameter B) as a function of ozone concentration was used as a measure of plant sensitivity. Applying this ranking scale, ponderosa pine, eucalyptus and soybean were the most sensitive species; holly, squash and marigold were least sensitive. There was a good correlation between ozone-induced stress ethylene production and foliar injury. However, the coefficient of variability associated with the ethylene determination was substantially less than with the visual injury estimate. The production of stress-induced ethylene generally lasted for less than 48 h following exposure. The measurement of stress ethylene appeared to be a fast, reliable, sensitive and reproducible technique to measure ozone stress on plants.

INTRODUCTION

Plants produce low levels of hormonal ethylene under normal conditions but when subjected to environmental stresses, ethylene production increases. This ethylene production following stress is frequently referred to as wound or stress-induced ethylene and injured, not dead tissue, is the site of the stress-induced ethylene production (Abeles, 1973). The enzyme complex that produces hormonal ethylene is apparently also responsible for the production of stress ethylene (Abeles and Abeles, 1972; Abeles, 1973).

In vegetation, stress ethylene can be induced by ozone (Craker, 1971; Abeles and Abeles, 1972; Uno and Miyake, 1972). Ozone-induced ethylene production peaked at 2–4 h following exposure and the amount of stress ethylene produced was in a physiologically active concentration (Craker, 1971; Uno and Miyake, 1972). Craker (1971) has proposed that some of the plant responses to ozone are the consequences of the stress ethylene produced by injured tissue. The primary action of ozone at the cellular level is probably to increase membrane permeability and allow ions and osmotic water to escape from the tissue (Evans and Ting, 1974; Heath *et al.*, 1974). This loss of osmotic water could cause cellular water stress and induce the production of stress ethylene as has been reported for water-stressed plants (El-Beltagy and Hall, 1974; Wright, 1974).

The increase in stress-induced ethylene was related to the intensity of several applied stresses. Maxie *et al.* (1965) reported that increasing the dosage of gamma radiation increased the production of stress ethylene. Shain and Wheeler (1975) proposed the measurement of stress-induced ethylene as a rapid method for assaying bacterial toxins. Stress ethylene production was also proportional to the number and size of foliar lesions induced by tobacco-mosaic virus (Nakagaki *et al.*, 1970).

The objectives of this study were: to determine if the production of ozone-induced stress ethylene could be used to determine differences in plant sensitivity to ozone; to determine if the production of ozone-induced stress ethylene was a sensitive and reliable method of measuring ozone stress on vegetation; to determine the duration of ozone-induced stress ethylene production; and to determine if the production of ozone-induced stress ethylene was correlated with the amount of visual foliar injury.

MATERIALS AND METHODS

Most plants tested in this study (Table 1) were grown from seed. The lilies which were grown from bulbs and the pines, rhododendrons, holly, kinnikinnick, rose, and potato were grown from cuttings or obtained from nurseries. The potting media was a mixture (1:2; V:V) of jiffy-mix† perlite. Plants were cultured in 10 cm dia. pots in the greenhouse at maximum day/night temperatures of 26 ± 3 and 18 ± 2 C, respectively. Sunlight was supplemented and the light period was extended to 16 h per day with light from HID sodium vapor lamps yielding an intensity of 350–600 μ einsteins $m^{-2} s^{-1}$ (400–700 nm) at plant height.

Plant exposures were conducted in modified greenhouse exposure chambers (Heck *et al.*, 1968) at the same conditions as the plants were grown. At the time of exposure, the plants depending on species varied in age from 3 weeks to 18 months. Ozone produced by u.v. radiation of air was metered into the exposure chambers and measured coulometrically with a Mast† ozone meter calibrated daily with known concetrations of ozone. The plants were

* To whom correspondence should be addressed.

† Mention of a proprietary product does not constitute a guarantee of warranty of the product by the Environmental Protection Agency and does not imply its approval to the exclusion of other products that may be suitable.

A.E. 10/11—D

usually exposed to either 0, 0.25, 0.50, or 0.75 ± 0.02 $\mu l\ l^{-1}$ for 4 h. Immediately following exposure, each plant and pot were enclosed in individual 2.7 l. polyethylene bags (1 mil) supported with wire frames which held the bag away from the foliage and provided a relatively constant volume. The enclosed plants were usually incubated at 26°C for 22 h without light. Preliminary studies had shown that dark incubation increased the production of stress-induced ethylene. Under these conditions, up to 10% of the stress ethylene could diffuse through the polyethylene film during incubation. Ethylene was sampled from the bag surrounding the plant by inserting a needle of a 1 ml glass syringe through the bag. When the ethylene was sampled there was no evidence of leaf wilt. Foliar injury was assessed visually in 5% increments as the percentage of the plant leaf area exhibiting ozone injury, approx. 72 h following exposure.

Ethylene was separated using a 1.83 m × 3.2 mm stainless steel column of chromosob 104 (80/100 mesh) at 100°C using helium (40 $cm^3\ min^{-1}$) as a carrier gas and measured with a flame ionization detector. Injector and manifold temperatures were maintained at 200°C. The presence of ethylene in the bag was confirmed by co-chromatography with standard ethylene on four different supports (chromosob 104, porapak Q; porapak R and porapak N) over the range of 60–150°C and by absorption of the ethylene in bromine water or aqueous permanganate (Crippen, 1973).

Early studies indicated that the variance of the ethylene measurements increased with the ethylene concentration. Therefore, the data were transformed to their respective natural logarithms to stabilize the variability (Snedecor and Cochran, 1968). All statistical calculations were performed on the transformed data.

RESULTS

The amount of ethylene that accumulated in the bag following plant exposure was influenced by both the amount of applied ozone and the plant species (Fig. 1). The measured stress ethylene evolved by plants exposed to 0.75 $\mu l\ l^{-1}$ ozone as compared to the control, increased by 13, 4 and 1.5 X for pine, tomato, and corn, respectively.

The increase in stress-induced ethylene in relation to applied ozone can be modeled using the following ln-linear equation: ln [ethylene concentration, nl l^{-1}] = ln A + B [ozone concentration, $\mu l\ l^{-1}$]. In this equation, the parameters A and B have the following interpretations: A is an estimate of the ethylene produced by the non-ozone stressed control plants and B is the slope parameter which is a measure of the increase in stress-induced ethylene production as a function of applied ozone. The slope parameter B is a measure of the responsiveness of the plant tissue to ozone stress as measured by the rate of stress ethylene production.

The slope parameter B, as a measure of the plant responsiveness to ozone stress, should also measure relative plant sensitivity to ozone. The slope parameter was computed for each species and/or cultivar and the plants were ranked according to their relative ozone sensitivity (Table 1). The data in Table 1 are the composite results from groups of plant species exposed over a several month period. Ozone enhanced the production of stress-induced ethylene

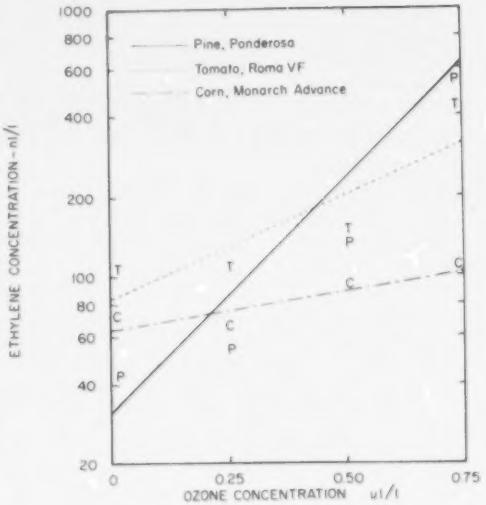

Fig. 1. The increase in ethylene concentration as a function of plant species and ozone concentration. Plants were exposed to ozone for 4 h and then placed in polyethylene bags and incubated for 22 h of incubation at 26°C. Ethylene concentration in the bag was then determined. Each mean was based on 12 observations. The slope parameter estimates for each species are listed in Table 1.

in all plant species. A large slope parameter indicated that the plants were sensitive; a small slope indicated that the plants were less sensitive to ozone as measured by ethylene evolution. Of the species tested ponderosa pine, eucalyptus, and soybean were most sensitive, holly, squash, and marigold were least sensitive. The slope parameter also indicated that tomato, begonia and lupine cultivars differed in ozone sensitivity (Table 1).

To serve as a reliable measure of plant sensitivity, the slope parameter B should be reproducible, with minimum variability. The reproductibility of the slope parameter was tested by conducting independent exposures on different plants of the same species to ozone over a period of several months and estimating the slope parameter B. Based on repeated trials, the slope parameter estimates for soybean, pine, and potato were all within their respective 95% confidence interval estimates (Table 2), suggesting that the slope parameter estimates were reproducible. The slope parameter estimate for ponderosa pine in Table 2 was substantially less than the estimate in Table 1. However, the data in Table 1 were collected on plants that had just undergone needle elongation in the spring and were in a highly sensitive stage while the data in Table 2 were collected during the fall.

The variability of ethylene determinations was compared with visual injury assessments. The coefficients of variability were determined for the mean ethylene concentration and foliar injury (Table 1). The coefficients of variability for the ethylene concentrations ranged between 6 and 37% with a mean of

Table 1. The relative ozone sensitivity of various plant species as determined by the rate of stress ethylene evolution and compared to foliar injury

Plant species	Slope parameter* *B*	Visible† foliar injury (%)	Coefficient of variability (%)	
			Mean ethylene	Mean foliar injury
Pinus ponderosa, Laws. Pine 'Ponderosa'	3.46	8	17	320
Eucalyptus globulus, Labill. Eucalyptus 'Blue Gum'	2.79	26	13	90
Glycine max (L.) Merr. Soybean 'Dare'	2.62	32	9	65
Lupinus Lupine hybrid 'Little Lulu'	2.45	22	13	79
Eucalyptus cinera Eucalyptus 'Silver Dollar'	2.38	23	20	106
Lupinus hybrid Lupine 'Pages'	2.13	33	11	85
Lycopersicon esculentum, Mill Tomato 'Roma VF'	1.79	34	13	84
Avena sativa, L. Oat 'Amitty'	1.68	40	8	54
Triticum aestivum L. em Thell. Wheat 'Hyslop'	1.66	39	8	71
Rhododendron hybrid Rhododendron 'Virginia Richards'	1.64	11	13	211
Begonia semperflorens, Link & Otto Begonia 'Scarletta'	1.34	24	6	40
Rhododendron hybrid Rhododendron 'Ramapo'	1.28	4	20	105
Lilium asiatic hybrid Lily, Enchantment	1.16	25	13	146
Arctostaphylos Uva-ursi (L.) Spreng. Kinnikinnick 'Alaska'	1.10	3	14	151
Lupinus hybrid Lupine 'Chatelaine'	0.96	12	9	158
Rhododendron hybrid Rhododendron 'Vulcan'	0.81	1	22	544
Lycopersicon esculentum, Mill Tomato 'Heinz 1439'	0.78	18	16	136
Cucumis sativus, L. Cucumber 'Bravo'	0.76	14	7	84
Alnus rubra, Bong Red Alder	0.74	28	24	120
Pinus mugo, var. Mughus, Zenari Pine 'Mugo'	0.72	0	15	0
Zea Mays L. Corn 'Monarch Advance'	0.63	8	6	103
Lilium longifolium, Thumb. Easter Lily 'Ace'	0.54	0	15	0
Lupinus hybrid Lupine 'Nobel Maiden'	0.54	6	9	254
Begonia semperflorens, Link & Otto Begonia 'White Tauendschon'	0.42	15	6	59
Solanum tuberosum, L. Potato 'Russet Burbank'	0.31	0	15	0
Rosa hybrid Rose 'Mary Devour'	0.38	3	27	137
Ilex aquifolium, L. English Holly	0.20	0	37	0
Cucurbita pepo, Var. Condensa, Bailey Squash 'Summer Crookneck'	0.13	5	7	271
Tagetes erecta, L. Marigold 'Gold Galore'	0.12	6	12	411

* Each slope parameter was computed using observations from 36 to 48 plants. Plants were exposed to ozone concentrations ranging from 0.0 to 0.75 $\mu l\,l^{-1}$ for 4 h and incubated in the dark for 22 h.

† Foliar injury: expressed as average % visible foliar injury to plants exposed to 0.75 $\mu l\,l^{-1}$ ozone for 4 h.

Table 2. Repeatability of the ethylene evolution slope parameter *B* from multiple experiments*

Plant species	Slope parameter *B*	95% confidence interval on *B*	Foliar† injury (%)
Glycine max (L.) Merr.	2.62	0.58	32
Soybean, Dare	2.09	0.80	43
	2.06	0.71	52
Pinus ponderosa, Laws.	1.41	0.73	0
Ponderosa Pine	0.93	0.63	0
Solanum tuberosum L.	0.39	0.68	3
Potato, Russet Burbank	0.36	0.81	0

* Each slope parameter was calculated from separate experiments using 36–48 plants for each estimate.

† Foliar injury at the highest ozone concentration (0.75 $\mu l\,l^{-1}$ for 4 h).

14%, while the coefficients of variability for the mean injury ranged between 0 and 544% with a mean of 134%.

Visual determination of foliar injury is one of the most frequently used measures of ozone effects on plants. To determine the relationship between the slope parameter *B* and the maximum foliar injury, a scatter plot was made (Fig. 2). Species which had higher slope parameter estimates tended to also have larger amounts of foliar injury. A significant linear regression line relating foliar injury to the slope parameter illustrates this association (Fig. 2). The intercept of the regression equation was not significantly different than 0. This indicates that generally there was a good relationship between the slope parameter estimate and visual injury as a measure of plant response. However, for several species such as mugo pine, easter lily, potato, holly (Table 1) and ponderosa pine (Table 2) there were significant increases in stress ethylene without any visual injury. This suggests that the ethylene measurement was a more sensitive measure of plant response than visual unjury.

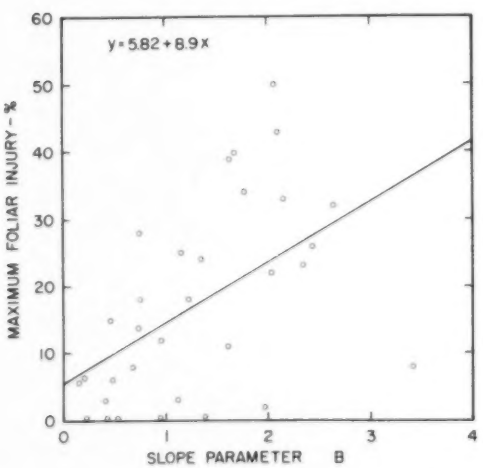

Fig. 2. A scatter plot illustrating the relationship between the ethylene evolution slope parameter *B* and foliar injury. The data for the slope parameters and ozone-induced foliar injury are from Tables 1 and 2.

To determine the duration of stress-induced ethylene production following exposure to ozone, soybeans were exposed to either 0, 0.4, or 0.8 $\mu l\,l^{-1}$ ozone for 2 h and then encapsulated at various times—0, 1, 2, 3 and 4 days after exposure. Each plant was encapsulated only once for 4 h. The 0.4 and 0.8 $\mu l\,l^{-1}$ treatments caused a significant increase in stress ethylene immediately following exposure (Fig. 3). However, within 24 h the stress-induced ethylene production from the 0.4 $\mu l\,l^{-1}$ treatment had returned to the control level and remained there for the duration of the study. The stress-induced ethylene production from the 0.8 $\mu l\,l^{-1}$ treatment remained high even 24 h following exposure and was significantly different from the control until 3 days following exposure. In a similar experiment with tomatoes (data not shown), the ozone treatments at both concentrations enhanced

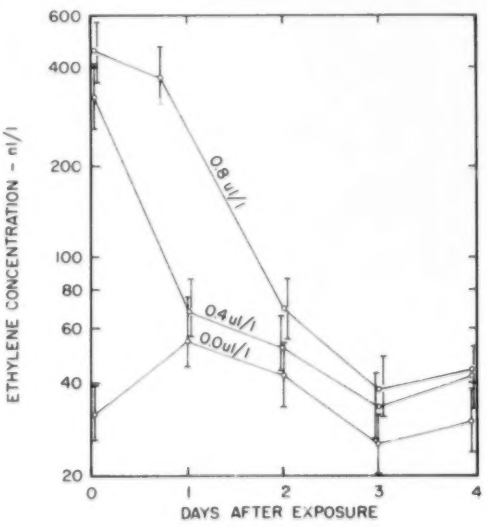

Fig. 3. The duration of stress ethylene production following a 2 h exposure to ozone. Soybean plants were exposed at day 0 and then encapsulated on subsequent days following exposure to determine ethylene production. The plants were incubated in the dark at 26 C for 4 h. Each mean was based on 6 observations. The bar about each mean is the standard error.

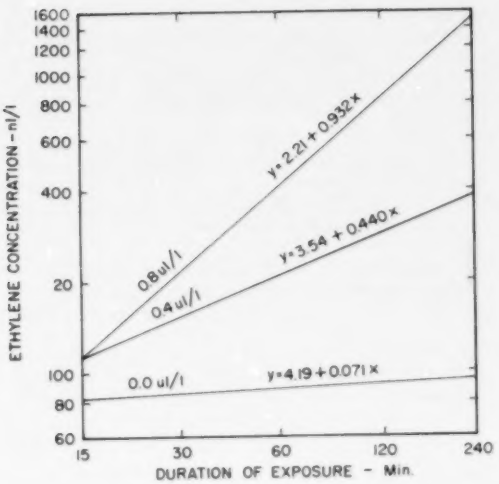

Fig. 4. The effect of exposure duration on the production of stress-induced ethylene from soybeans. Data from 40 plants were used to estimate each regression line. 95% confidence interval estimates on the parameters A and B are 1.23 and 0.232, respectively. The x and y variables of the regression equation are expressed the ln of the ethylene concentration and the ln of the duration of exposure in min. The plants were incubated under standard conditions (see Methods).

stress-induced ethylene production immediately following exposure, but within 1 day there was no significant difference in the ethylene production between the treated and control plants.

Previous studies suggested that the natural logarithm of the stress ethylene production was linearly related to the ozone concentration (Fig. 1; Table 1). To determine the influence of exposure duration on ethylene production, soybeans were exposed to either 0, 0.4 or 0.8 $\mu l\,l^{-1}$ ozone for 15–240 min. The logarithm of the stress ethylene concentration exhibited a linear increase when plotted against the logarithm of the exposure duration (Fig. 4). As little as a 15 min exposure to either 0.4 or 0.8 $\mu l\,l^{-1}$ ozone caused a 41% increase in stress ethylene. Longer exposures to either concentration resulted in greater increases in stress-induced ethylene.

DISCUSSION

The production of stress ethylene following an ozone exposure was similar to previous reports (Craker 1971; Abeles and Abeles 1972; and Uno and Miyake, 1972). However, these authors did not attempt to use stress-induced ethylene to quantify ozone-induced plant stress.

The increase in stress ethylene with an increase in applied ozone, suggested that the slope of the regression line would be a good measure of plant sensitivity to ozone stress. The slope parameter indicated the magnitude of increase in stress-induced ethylene production for a given increase in the ozone concentration. Thus, the slope parameter was a measure of tissue response to ozone stress. Also, the slope parameter in contrast to the actual ethylene concentration was independent of the amount of tissue used and therefore, can be used to compare the sensitivity of different species and/or cultivars.

Visual injury has been proposed as the most rapid and sensitive technique to measure the response of plants to acute pollution insults (Dunning and Heck, 1973). The visual assessment technique is more rapid than a measurement of stress ethylene, but it is also subjective. The measurement of stress ethylene can be standardized among researchers and laboratories, it exhibits less variability and is more reproducible than the visual procedure; and it exhibits a known statistical distribution. The induction of stress ethylene by ozone in the absence of visual injury suggests that the stress-induced ethylene measurement is also a more sensitive measure of the plant response to ozone than visual injury.

The sensitivity ranking of plant species and cultivars to ozone by the slope parameter B generally agrees with previous reports (Hill *et al.*, 1961; Heck and Tingey, 1971; Reinert *et al.*, 1972) using the visual techniques. However, in this study there were some differences in sensitivity because different plant cultivars were used than in the earlier studies.

These data and the work of Craker (1971) and Uno and Miyake (1972) indicate that the ozone induction of stress ethylene is a short-lived phenomenon of up to 48 h duration. The maximum production rate of stress ethylene occurred in the first 2–4 h period following exposure. This burst of stress ethylene following an ozone exposure suggests that ethylene is involved in the trigger mechanism of plant responses to ozone as proposed by Craker (1971).

Acknowledgements—The assistance and suggestions of Mr. Nelson Pettit of Northrop Services, Incorporated and Dr. Robert G. Linderman, USDA-ARS Corvallis, Oregon, were appreciated.

REFERENCES

Abeles F. B. (1973) *Ethylene in Plant Biology*. Academic Press, New York.

Abeles A. L. and Abeles F. B. (1972) Biochemical pathway of stress-induced ethylene. *Plant Physiol.* **50**, 496–498.

Craker L. E. (1971) Ethylene production from ozone injured plants. *Environ. Pollut.* **1**, 299–304.

Crippen R. C. (1973) *Identification of Organic Compounds with the Aid of Gas Chromatography*. McGraw-Hill, New York.

Dunning J. A. and Heck W. W. (1973) Response of pinto bean and tobacco to ozone as conditioned by light intensity and/or humidity. *Environ. Sci. Technol.* **7**, 824–826.

El-Beltagy A. S. and Hall M. H. (1974) Effect of water stress upon endogenous ethylene levels in *Vicia faba*. *New Phytol.* **73**, 47–60.

Evans L. S. and Ting I. P. (1974) Ozone sensitivity of leaves: Relationship to leaf water content, gas transfer resistance, and anatomical characteristics. *Am. J. Bot.* **61**, 592–597.

Heath R. L., Chimiklis P. and Frederick P. (1974) The role of potassium and lipids in ozone injury to plant membranes. In *Air Pollution Effects on Plant Growth* (Edited by Dugger M.) American Chemical Society Symposium Series Vol. 3, pp. 58–75.

Heck W. W., Dunning J. A. and Johnson H. (1968) Design of a simple plant exposure chamber. DHEW, National Center of Air Pollution Control Publ. APTD-68-6, Washington, DC.

Heck W. W. and Tingey D. T. (1971) Ozone. Time-concentration model to predict acute foliar injury. In *Proceedings Second International Clean Air Congress* (Edited by Englund H. M. and Berry W. T.), pp. 249–258. Academic Press, New York.

Hill A. C., Pack M. R., Treshow M., Downs R. J. and Transtrum L. G. (1961) Plant injury induced by ozone. *Phytopathology* **51,** 356–363.

Maxie E. C., Eaks I. L., Sommer N. F., Rae H. L. and El-Batal S. (1965) Effect of gamma radiation on rate of ethylene and carbon dioxide evolution by lemon fruit. *Plant Physiol.* **40,** 407–409.

Nakagaki Y., Hirai T. and Stahmann M. A. (1970) Ethylene production by detached leaves infected with tobacco-mosaic virus. *Virology* **40,** 1–9.

Reinert R. A., Tingey D. T. and Carter H. B. (1972) Sensitivity of tomato cultivars to ozone. *J. Am. Soc. Hort. Sci.* **97,** 149–151.

Shain L. and Wheeler H. (1975) Production of ethylene by oats resistant and susceptible to victorin. *Phytopathology* **65,** 88–89.

Snedecor G. W. and Cochran W. G. (1968) *Statistical Methods*, 6th Edn. The Iowa State University Press, Ames, IA.

Uno Y. and Miyake Y. (1972) Studies on physiological tobacco leaf spot (IV). Ethylene production on physiological tobacco leaf spot. *Bull. Utsunomiya Tob. Exp. Stn.* **11,** 23–27.

Wright M. (1974) The effect of chilling on ethylene production, membrane permeability and water loss of leaves at *Phaseolus vulgaris*. *Planta* **120,** 63–69.